

INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference 10288.204-WO		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)
International application No. PCT/DK 03/00474	International filing date (day/month/year) 04.07.2003	Priority date (day/month/year) 25.07.2002
International Patent Classification (IPC) or both national classification and IPC C12C7/00		
Applicant NOVOZYMES AS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority, (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 05.02.2004	Date of completion of this report 20.08.2004
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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/DK 03/00474**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-26 as originally filed

Claims, Numbers

1-14 as amended (together with any statement) under Art. 19 PCT

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

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III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10-11

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 10-11 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	6-7,12
	No: Claims	1-5, 8-9, 13-14
Inventive step (IS)	Yes: Claims	
	No: Claims	6-7, 12
Industrial applicability (IA)	Yes: Claims	1-14
	No: Claims	

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/DK 03/00474

- D1: US-A-4 788 066 (WITT PAUL R) 29 November 1988 (1988-11-29)
D2: GB-A-1 248 505 (CHEMISCHE FABRIEK NAARDEN) 6 October 1971
(1971-10-06)
D3: DE 23 20 425 A (BOEHRINGER SOHN INGELHEIM) 7 November 1974
(1974-11-07)

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The subject-matter of claims 10 and 11 comprises only a desideratum: no defined technical features are given in said claims which are necessary to obtain the desired effect (low concentration of dimethyl sulfide or trans-2-nonenal). The term "relative to the level in a wort or beer produced by the standard Congress mashing process" is meaningless per se without indicating a precise **reference concentration** with respect to said compounds.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1). Novelty (Article 33.2 PCT)

Having regard to **D1-D3** cited in the International Search Report the subject-matter of claims 1-5, 8-9, and 13-14 lacks novelty.

D1 describes in the claims and example 1 already a process for forming a mash comprising between 5% and 100% of coarsely ground barley malt. An amylase and cellulase (Laminex) were added and mashing continued for an additional 30 minutes at 78°C. The hot mash was filtered in order to separate the wort from the grains. The wort was fermented with yeast to obtain a beer. Having regard to **D1** the subject-matter of claims 1, 3, 5, 8-9 and 13-14 lacks novelty.

D2 describes in the claims and the examples already a process for forming a mash comprising between 5% and 100% of barley. An alpha-amylase, cellulase and protease was added and the temperature was adjusted to 87°C in 15 minutes and

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held for an additional 30 minutes at this temperature (example 1). A saccharifying enzyme (i.e. a maltose generating enzyme) was also added to the mash. The mash was filtered in order to separate the wort from the grains. Thus, with respect to **D2** the subject-matter of claims 1-4, 8, and 13 lacks novelty.

A similar process as described in **D2** and performed at a temperature between 67° and 73° C has been disclosed in **D3**.

2). Inventive step (Article 33.3 PCT)

With respect to documents **D1-D3** the subject-matter of claims 6-7 and 12 is considered to be novel but lacks the required inventive step. The subject-matter of said claims concerns only minor modifications of the processes for producing wort/beer already described in the above mentioned prior art, which were within the easy reach of the skilled person.

AMENDED CLAIMS

[received by the International Bureau on 15 January 2004 (15.01.04);
new claims 13 and 14 added; original claims 9-14, 18, 19, and 21-25 deleted;
remaining claims renumbered (2 pages)]

- 1) A process for production of a wort, comprising;
 - a) forming a mash comprising between 5% and 100% barley malt (w/w of the grist);
 - b) prior to, during or after a) adding a cellulase (E.C. 3.2.1.4);
 - c) attaining within 15 minutes of a) an initial incubation temperature of at least 70°C;
 - d) following c) incubating the mash at a temperature of at least 70°C for a period of time sufficient to achieve an extract recovery of at least 80%; and,
 - e) separating the wort from the spent grains.
- 2) The process according to claim 1, wherein further a protease (E.C. 3.4.) is added.
- 3) The process according to any of the proceeding claims, wherein further an alpha-amylase (E.C. 3.2.1.1) is added.
- 4) The process according to any of the proceeding claims, wherein further a maltose generating enzyme is added.
- 5) A process for production of a beer, comprising obtaining the wort of the process of any of claims 1-4, fermenting said wort with a yeast, and obtaining a beer.
- 6) A process for production of a beer, comprising obtaining the wort of the process of any of claims 1-4, combining said wort with a second wort, fermenting the combined wort with a yeast, and obtaining a beer.
- 7) A process for production of a beer, comprising obtaining the wort of the process of any of claims 1-4, fermenting said wort with a yeast, combining the fermented wort with a fermented second wort, and obtaining a beer.
- 8) The process according to any of the proceeding claims, wherein at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of the wort defined in claim 1 is barley malt.
- 9) The process of any of claims 5-8, wherein the beer is ales, strong ales, bitters, stouts, porters, lagers, export beers, malt liquors, barley wine, happoushu, high-alcohol beer,

low-alcohol beer, low-calorie beer or light beer.

- 10) The process of any of the proceeding claims, wherein the concentration of dimethyl sulfide in the wort or the beer is reduced, such as by 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% relative to the level in a wort or a beer produced by the standard Congress mashing process.
- 11) The process of any of the proceeding claims, wherein the concentration of trans-2-nonenal of the wort or the beer is reduced, such as by at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% relative to the level in a wort or a beer produced by the standard Congress mashing process.
- 12) The process according to any of claims 4-11; wherein the maltose generating enzyme is a beta-amylase (E.C. 3.2.1.2) or a maltogenic alpha-amylase (E.C. 3.2.1.133).
- 13) The wort produced by the process of any of claims 1-12.
- 14) The beer produced by the process of any of claims 5-12.

- 1) A process for production of a wort, comprising;
 - a) forming a mash comprising between 5% and 100% barley malt (w/w of the grist);
 - b) prior to, during or after a) adding a cellulase (E.C. 3.2.1.4);
 - 5 c) attaining within 15 minutes of a) an initial incubation temperature of at least 70°C;
 - d) following c) incubating the mash at a temperature of at least 70°C for a period of time sufficient to achieve an extract recovery of at least 80%; and,
 - e) separating the wort from the spent grains.
- 2) The process according to claim 1, wherein further a protease (E.C. 3.4.) is added.
- 10 3) The process according to any of the proceeding claims, wherein further an alpha-amylase (E.C. 3.2.1.1) is added.
- 4) The process according to any of the proceeding claims, wherein further a maltose generating enzyme is added.
- 5) A process for production of a beer, comprising obtaining the wort of the process of any of
15 claims 1-4, fermenting said wort with a yeast, and obtaining a beer.
- 6) A process for production of a beer, comprising obtaining the wort of the process of any of claims 1-4, combining said wort with a second wort, fermenting the combined wort with a yeast, and obtaining a beer.
- 7) A process for production of a beer, comprising obtaining the wort of the process of any of
20 claims 1-4, fermenting said wort with a yeast, combining the fermented wort with a fermented second wort, and obtaining a beer.
- 8) The process according to any of the proceeding claims, wherein at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w) of the grist of
25 the wort defined in claim 1 is barley malt.
- 9) The process according to any of the proceeding claims, wherein the initial incubation tem-

perature is attained within 14 minutes, or more preferably within 13 minutes, such as within, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 minutes, or even more preferably within 1 minute after the mash forming, or most preferably, is attained at the mash forming.

- 5 10) The process according to any of the proceeding claims, wherein the initial incubation temperature is at least 71°C, preferably at least 72°C, more preferably at least 73°C, even more preferably at least 74°C, or most preferably at least 75°C, such as at least 76°C, at least 77°C, at least 78°C, at least 79°C, at least 80°C, at least 81°C, or at least 82°C.
- 10 11) The process according to any of the proceeding claims, wherein the temperature is increased and/or decreased during the incubation with at least 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C or preferably with at least 10°C, or more preferably with at least 12°C, such as at least 15°C.
- 15 12) The process according to any of the proceeding claims, wherein the incubation under step d) comprises maintaining the mash at a temperature of at least 75°C, preferably at least 76°C, more preferably at least 77°C, even more preferably at least 78°C, such as at least 79°C, at least 80°C, at least 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C or at least 90°C for a period of at least 1 minute, preferably for at least 5 minutes, more preferably for at least 15 minutes, even more preferably for at least 20 minutes, such as for at least 30 minutes, at least 40 minutes, at least 50 minutes, at least 60 minutes, at least 90 minutes, or at least 110 minutes.
- 20 13) The process according to any of the proceeding claims, wherein the duration of the incubation period is between 15 minutes and 2 ½ hour, such as at least 30 minutes, at least 45 minutes, at least 1 hour, at least 1¼ hour, at least 1½ hour, at least 1¾ hour or at least 2 hours.
- 25 14) The process of any of the proceeding claims, wherein the extract recovery from the mash defined in claim 1 is at least 81%, preferably at least 82%, more preferably at least 83%, even more preferably at least 84%, or most preferably at least 85%, such as at least 86%.
- 15) The process of any of claims 5-14, wherein the beer is ales, strong ales, bitters, stouts, porters, lagers, export beers, malt liquors, barley wine, happoushu, high-alcohol beer, low-alcohol beer, low-calorie beer or light beer.

6) The process of any of the proceeding claims, wherein the concentration of dimethyl sulfide in the wort or the beer is reduced, such as by 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% relative to the level in a wort or a beer produced by the standard Congress mashing process.

17) The process of any of the proceeding claims, wherein the concentration of trans-2-nonenal of the wort or the beer is reduced, such as by at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% relative to the level in a wort or a beer produced by the standard Congress mashing process.

18) The process of any of claims 3-17, wherein the alpha-amylase is derived from a *Bacillus* sp.

19) The process of any of claims 3-18, wherein the *Bacillus* alpha-amylase has an amino acid sequence having at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% homology to SEQ ID NO:4 in WO99/19467.

20) The process according to any of claims 4-19; wherein the maltose generating enzyme is a beta-amylase (E.C. 3.2.1.2) or a maltogenic alpha-amylase (E.C. 3.2.1.133).

21) The process according to any of claims 4-20, wherein the maltogenic alpha-amylase is derived from *Bacillus*, preferably from *B. stearothermophilus*.

22) The process according to any of the claims 4-21, wherein the maltogenic alpha-amylase has at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% homology to the amino acid sequence shown as SEQ ID NO:1 in US 6,162,628.

23) The process according to any of the proceeding claims, wherein the protease is derived from *Bacillus amyloliquefaciens* and has at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% homology to the amino acid sequence obtainable at Swissprot as Accession No. P06832.

24) The process according to any of the proceeding claims, wherein the protease has at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% homology to the sequence disclosed as amino acids no. 1-177 of SEQ.ID.NO:2 in

Danish patent applications PA 2001 01821 and PA 2002 00005.

25) The process according to any of the proceeding claims, wherein the cellulase is an endo-glucanase, such as an endo-1,4-beta-glucanase having at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99% homology to the amino acid sequence disclosed as amino acids no. 1-177 of SEQ.ID.NO:2 in Danish patent application PA 2002 00130.

26) The wort produced by the process of any of claims 1-25.

27) The beer produced by the process of any of claims 5-25.